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Oxime Derivatives of Sordaricin as Potent Antifungal Agents

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Abstract—Oxime derivatives of the sordarin aglycone have been identified as potent antifungal agents. The in vitro spectrum of activity includes coverage against *Candida albicans* and *Candida glabrata* with MICs as low as 0.06 μg/mL. The antifungal activity was established to be exquisitely sensitive to the spatial orientation of the lipophilic side chains.

Invasive fungi have in recent decades emerged as important pathogens, in particular among the growing and increasingly diverse population of immunocompromised patients. *Candida albicans*, for instance, is the primary cause of fungal infections in neutropenic and solid-organ transplant patients, and is also implicated in oral candidiasis cases typically found in HIV-infected individuals.[1,2] Despite the growing medical need, however, few existing therapies with suitable safety and efficacy profiles are available for the treatment of systemic fungal infections. Moreover, the emergence of fungal pathogens resistant to current chemotherapies further increases the demand for the discovery and development of novel agents.

The natural product sordarin (**1**) was discovered in 1971 as a metabolite of *Sordaria araneosa* and identified as a potent antifungal compound (Fig. 1).[3] Interest in this diterpene glycoside has recently been sparked by the identification of its cellular target in fungal organisms, the translational elongation factor EF-2.[4,5] Despite the high sequence homology between fungal and human EF-2 (85%), sordarin is able to exert a specific effect on fungal EF-2 by binding with high affinity to the ribosome/EF-2 complex and blocking translocation.[6] The selective inhibition of fungal protein synthesis thus became an attractive target for the development of new antifungal agents that were mechanistically distinct from existing therapies.

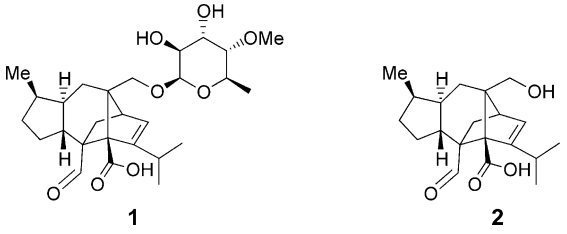

Figure 1.

An interesting development in the exploration of this natural product was the report by Tse et al. that when the sordarin aglycone, known as sordaricin (**2**), was functionalized with lipophilic side chains, the antifungal activity against *Saccharomyces cerevisiae* was markedly improved relative to both **1** and **2**.[7] As part of our efforts to discover novel agents for the treatment of systemic fungal infections, we became interested in **2** as a versatile template for the synthesis of sordaricin analogues. In this communication, we report the synthesis of novel sordaricin oxime derivatives and their potent in vitro activity against several *Candida* pathogens.

From a synthetic standpoint, we envisioned a differentially-protected dialdehyde version of **2**[8] as a reasonable launching point for the exploration of diverse structural architecture. To this end, routine protection of the carboxylic acid and aldehyde groups in **2** was followed by oxidation of the primary alcohol to afford the monoprotected dialdehyde core **4** (Scheme 1). Condensation of **4** with hydroxylamine hydrochloride afforded the oxime derivative **5**.

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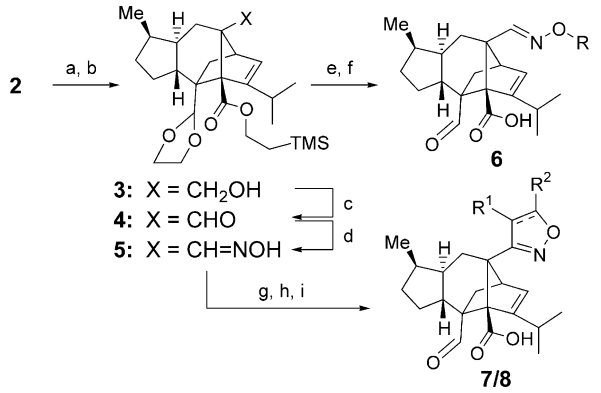


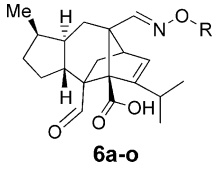
Scheme 1. Preparation of sordaricin oxime derivatives: (a) *O*-[2-(trimethylsilyl)ethyl] diisopropyl isourea, THF, reflux; (b) ethylene glycol, PPTS, MeOH; (c) TPAP, NMO, molecular sieves, CH_2Cl_2; (d) hydroxylamine hydrochloride, pyridine, rt; (e) R-X, NaH, DMF; (f) 1 N HCl, MeOH; (g) *N*-bromosuccinimide, pyridine, CH_2Cl_2; (h) Et_3N, alkene/alkyne, benzene, reflux; (i) TBAF, THF, then 1 N HCl.

The oxime intermediate **5** could then be elaborated in several fashions. Reaction of the oxime with a variety of electrophiles and NaH in DMF afforded the *O*-alkylated products with concomitant cleavage of the 2-(trimethylsilyl)ethyl ester protecting group.[9] Selective acidic hydrolysis of the ethylene ketal in the presence of the oxime ether afforded the final targets **6a–o**,[10,11] which proved to be robust under typical assay conditions.[12] Alternatively, the oxime could be oxidized with *N*-bromosuccinimide and then converted in situ to the nitrile oxide. Reaction of this intermediate with a variety of alkenes and alkynes followed by standard deprotection steps generated the conformationally-restricted [3 + 2] cycloadducts **7a–n**[13] and **8a–b**.

The acyclic oxime ethers **6a–o** were tested for fungal growth inhibition in *C. albicans*, *Candida glabrata*, *Cryptococcus neoformans*, and several other pathogens (Table 1).[14] In general, incorporating lipophilic functionality in the oxime ether side chain led to increased whole cell activity relative to the natural product (**1**). The most potent analogue in this series (**6f**, R = *n*-pentyl) had an MIC against both *Candida* pathogens of 0.06 μg/mL and a therapeutic index (CC_{50}/MIC) of 92. Against non-*Candida* pathogens, compound **6f** was the only derivative to register an MIC versus *C. neoformans* (MIC = 64 μg/mL), while no activity was observed for compounds **6a–o** against *Aspergillus fumigatus* and other filamentous fungi. A slight decrease in anti-*Candida* activity was observed with branched side chain derivatives (**6e** and **6g–i**), which suggests that lipophilicity alone is not sufficient to explain the excellent anti-*Candida* activity of **6f**. Aromatic (**6m–n**) and heteroaromatic (**6o**) oxime ethers were also poor inhibitors of fungal growth.

The isoxazolines **7a–n** and isoxazoles **8a–b** provided a unique opportunity to conformationally restrict the oxime side chain appendages while also rendering more stable oxime analogues (Table 2). Consistent with our earlier SAR observations, bicyclic sordaricin derivatives containing polar substituents such as tertiary amines (**7b** and **7e**), nitrile (**7d**), and primary alcohols (**7f** and **8a**) were ineffective against all fungal pathogens examined. Although [3 + 2] cycloadducts with more lipophilic substitution were slightly better inhibitors (i.e., **7h–m**), this was true only for compounds derived from monosubstituted dipolariphiles. The product derived from cyclohexene (**7c**), for instance, had no measurable activity against *C. albicans* but was moderately effective against *C. glabrata* (MIC = 1 μg/mL).

Table 1. In vitro activity of sordaricin oxime ethers **6a–o**

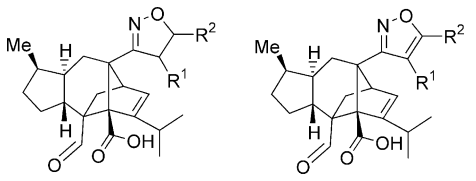


6a-o

Compd	R	MIC[a] (μg/mL)		CC_{50}[b] (μg/mL)	CC_{50}/MIC (μg/mL)
		C. albicans	*C. glabrata*	Cytotoxicity	Therapeutic Index
1	—	16	> 128	167	10
2	—	> 128	> 128	nd[c]	—
6a	2-Tetrahydropyranyl	0.5	1	33	66
6b	(2-Tetrahydropyranyl)methyl	2	8	9	4.5
6c	2-(2-MeO-ethoxy)ethyl	8	128	46	5.7
6d	2-Ethoxyethyl	16	16	22	1.4
6e	Cyclohexylmethyl	0.25	1	0.6	2.4
6f	*n*-Pentyl	0.06	0.06	5.5	92
6g	*R*-2-Methylbutyl	0.25	0.5	2.7	11
6h	*i*-Butyl	0.5	0.5	35	70
6i	*i*-Pentyl	0.5	1	4	8
6j	4,4,4-Trifluorobutyl	1	4	1.9	1.9
6k	3-Methyl-2-butenyl	1	1	4.5	4.5
6l	2-Butynyl	8	32	16	2
6m	2-Chlorobenzyl	2	8	1.4	0.7
6n	2-Methylbenzyl	16	16	nd	—
6o	[3,5-di-Me-isoxazol-4-yl]methyl	> 128	128	nd	—

[a]MIC value defined as the lowest drug concentration required to inhibit 90–100% visible growth relative to controls.
[b]CC_{50} value measured against Hep 2 cells.
[c]nd, not determined.

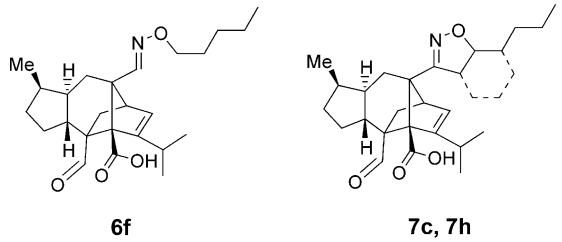
Table 2. In vitro activity of sordaricin isoxazolines **7a–n** and isoxazoles **8a–b**



7a-n **8a-b**

Compd	R^1	R^2	MIC (μg/mL)	
			C. albicans	*C. glabrata*
7a		Tetrahydrofuranyl	>128	8
7b		*N*-Benzyl pyrrolidinyl	>128	>128
7c		Cyclohexyl	>128	1
7d	H	$-CH_2CN$	128	128
7e	H	*N*-Morpholino methyl	16	>128
7f	H	$-CH_2OH$	>128	>128
8a	H	$-CH_2OH$	>128	>128
7g	H	$-CO_2Et$	8	4
7h	H	$-(CH_2)_3CH_3$	2	2
7i	H	$-O(CH_2)_3CH_3$	1	8
7j	H	*i*-Pentyl	1	4
7k	H	2,2-Dimethylpropyl	2	1
7l	H	2,2-Dimethylbutyl	4	1
7m	H	Cyclohexyl	2	2
7n	H	Phenyl	16	2
8b	H	Phenyl	8	0.25

The disparate activities of the acyclic oxime ether **6f** and the cycloadduct **7h** further emphasize the sensitive steric interaction between the sordaricin analogues and the EF-2/ribosome assembly. Even though both oxime ethers maintain a similar *trans* relationship between the C–C and N–O bonds, the *n*-pentyl side chain in **6f** can apparently more readily adopt the optimal conformation for proper EF-2/ribosome interaction, while the aliphatic chain in **7h** is locked via the isoxazoline ring (Fig. 2). Additional conformational restriction via the fused bicyclic ring system (**7c**) completely prevents the alkyl side chain from accessing the same space available to compound **6f**. Taken together with our earlier observation in the acyclic series **6a–o** that side chain branching diminishes potency, we can only speculate that a narrow, hydrophobic pocket exists in the ribosomal assembly that is extremely sensitive to slight perturbations in drug structure.[15]



6f **7c, 7h**

Figure 2.

In summary, we have developed a novel sordaricin dialdehyde core that serves as a multifunctional template for analogue synthesis. The discovery of potent oxime ether analogues establishes that the antifungal activity of sordaricin derivatives includes clinically relevant pathogens such as *C. albicans* and *C. glabrata*. The apparent sensitivity of the EF-2/ribosome assembly to the structure and conformation of the side chain functionality suggests that a more detailed understanding of the active site interactions is required.[16]

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8. For the preparation of **2** from sordarin, see: Serrano-Wu, M. H.; St. Laurent, D. R.; Chen, Y.; Lam, K.; Matson, J. A.; Mazzucco, C. E.; Tully, T.; Wong, H. S.; Vyas, D.; Balasubramanian, B. Manuscript submitted for publication.
9. Serrano-Wu, M. H.; Regueiro-Ren, A.; St. Laurent, D. R.; Carroll, T. M.; Balasubramanian, B. *Tetrahedron Lett.* **2001**, *42*, 8593.
10. A single oxime isomer was observed for intermediate **5** and compounds **6a–o**. Both the chemical shift (7.3 ppm) and NOE experiments suggest the oxime derivatives exist in the (E)-geometry.
11. **Representative procedure for *O*-alkylation of 5.** Unwashed NaH (5.0 equiv) was added in one portion to a stirred DMF solution of the oxime **5** (1.0 equiv), Bu_4NI (1.0 equiv) and the electrophile (1.1 equiv) under nitrogen at room temperature. The mixture was stirred at room temperature overnight and then partitioned between EtOAc and saturated NH_4Cl solution. The organic phase was separated, washed with saturated $NaHCO_3$ solution and brine, dried, and concentrated. The residue was then dissolved in MeOH and cooled to 0 °C and treated with an equal volume of 1 N HCl. After stirring at 0 °C for 0.5 h, the mixture was allowed to warm to ambient temperature over 2 h. Extractive workup followed by pre-

parative TLC purification (5% methanol in chloroform) afforded the fully deprotected sordaricin derivative. All compounds were characterized by ^{1}H NMR, LC–MS, and LR–MS. For example, analytical data for **6f**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.96 (1H, s), 7.34 (1H, s), 6.02 (1H, m), 4.01 (2H, m), 2.78 (1H, m), 2.27 (2H, m), 2.09 (2H, m), 1.91 (2H, m), 1.61–1.81 (6H, m), 1.28 (7H, m), 1.00 (3H, d, $J = 6.7$ Hz), 0.98 (3H, d, $J = 6.7$ Hz), 0.89 (3H, m), 0.82 (3H, d, $J = 6.7$ Hz). LRMS (ESI, m/z, M–H$^-$) 414.

12. For example, oxime ether **6g** was stable up to 72 h at pH 6.5. However under more acidic conditions (pH 2), only 13% of the compound remained after 24 h.

13. **Representative procedure for [3 + 2] cycloaddition of 5.** To a solution of **5** in dichloromethane was added 2.0 equiv of pyridine followed by 1.3 equiv of *N*-bromosuccinimide as a solid. After 0.5 h, TLC analysis showed clean formation of the bromo oxime intermediate. To this crude reaction mixture was added an equal volume of benzene followed by triethylamine (3.0 equiv) and 10.0 equiv of the dipolariphile. The reaction was heated to 80 °C overnight. Following extractive workup, the crude residue was dissolved in THF and treated with 1.5 equiv of TBAF. After 4 h, an equal volume of 1 N HCl was added, and the reaction was monitored by TLC. Neutralization and extractive workup afforded the deprotected sordaricin derivatives which were purified by preparative TLC. Compounds **7a**–**n** were synthesized and tested as a 1:1 mixture of diastereomers.

14. The fungal strains used in this study were obtained from the American Type Culture Collection. MICs were determined according to NCCLS standards.

15. Interestingly, Justice and co-workers have recently identified tyrosine 521 and serine 523 as important amino acids for sordarin sensitivity. See: Shastry, M.; Nielsen, J.; Ku, T.; Hsu, M.; Liberator, P.; Anderson, J.; Schmatz, D.; Justice, M. C. *Microbiology* **2001**, *147*, 383.

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